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Identification of red rice, rice, and hybrid populations using microsatellite markers

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Red rice is a major weed in rice production in the southern United States. Red rice and rice intercross because they are the same species. Our objectives were to determine the genetic diversity represented by accessions of red rice and to identify DNA markers that might be useful in identifying hybrids between red and cultivated rice. Red rice accessions were collected from Arkansas and other rice-producing states. Seventy-nine red rice accessions, 10 known or putative hybrid derivatives of red rice and cultivated rice (RC hybrids), and seven rice cultivars were analyzed using microsatellite DNA markers developed for cultivated rice. Microsatellite markers differentiated awned and awnless red rice accessions, six of the seven rice cultivars, and all 10 RC hybrids tested. Thus, these markers were useful in identifying red rice types and RC hybrids.

Nomenclature: Red rice, *Oryza sativa* L. ORYSA; rice, *Oryza sativa* L.

Key words: Accession, biotype, DNA fingerprinting, genetic distance, hybrid, outcrossing, polymorphism, simple sequence repeat, SSR.

Red rice is the most troublesome weed in rice cropping systems in the southern United States (Bridges and Baumann 1992; Dowler 1997; Webster 2000). Red rice is of the same genus and species as cultivated rice (Hoagland and Paul 1978), making it particularly difficult to control with herbicides or other methods in drill-seeded rice systems. Rice weed specialists (F. Baldwin, J. Chandler, A. Kendig, A. Klosterboer, M. Kurtz, S. Linscombe, and E. Webster, personal communication) estimated that red rice infestations cause significant economic losses in 15 to 65% of the rice acreage in states of the Mississippi delta and the Gulf coast. Losses from red rice in the United States have been estimated at $50 million annually (Smith 1979). More recently, the total annual losses from all weeds in rice produced in the South were estimated at $45 and $640 million, respectively, using "best management practices" that did or did not include herbicides (Bridges and Baumann 1992).

Rice is one of the most important world crops, providing the main food source for more than half of the people on earth (Singh and Khush 2000). It is a useful and simple genetic model for other cereal crops (Izawa and Shimamoto 1996) because of its small (2.02 pg) genome (Bennett and Leitch 1997). Rice is a diploid ($2n = 24$) in the "AA" genome group of *Oryza*. *Oryza sativa* plant types (e.g., red rice and cultivated rice) intercross among themselves and with other species in the AA group (Singh and Khush 2000; Sitch 1990).

Rice cultivars are primarily self-pollinated, averaging about 0.5% (range 0 to 3.4%) outcrossing in four U.S. locations over a 4- to 6-yr period (Beachell et al. 1938). Rice and red rice also appear to interbreed at similar low rates (Langevin et al. 1990; Mitten et al. 2001; Rutger 1992; Sanders et al. 2000), but the precise gene flow dynamics between rice and red rice under field situations are not well understood. Intercrossing between red rice and rice complicates the identification of red rice populations as well as control strategies. Concerns include red rice types acquiring resistance naturally or becoming resistant through crossing with herbicide-resistant rice cultivars (Oard et al. 1996, 2000).

Although primarily regarded as a pest, some red rice accessions are known to be disease resistant (Jia et al. 2001; Lee et al. 2000) and may be useful sources of genes for disease resistance in cultivated rice. Introgression of disease resistance and stress tolerance traits from wild germ plasm is a well-established breeding strategy for rice improvement (Sitch 1990). Useful genes for yield and other agronomic characteristics have been identified from wild relatives such as *O. rufipogon* (Xiao et al. 1996).

Red rice and rice have similar morphologies, allowing them to coexist in rice fields over time. Red rice types with wide-ranging characteristics, including competitive ability, tillering capacity, flowering date, seed dormancy, and tolerance to chilling and to several herbicides, have been found in southern U.S. rice fields (Estorninos 2000; Gealy and Black 1998, 1999; Gealy et al. 1999; Noldin et al. 1999a, 1999b). The regional distribution and genetics of red rice populations in South Korea have been characterized extensively (Cho et al. 1995; Suh et al. 1992, 1997). The genetic interrelationships among a limited number of U.S. red rice populations have recently been investigated using molecular techniques (Estorninos et al. 2000; Vaughan et al. 2001). An improved understanding of the genetics of red rice populations in the United States and their interrelatedness with cultivated rice is needed because of the importance of red rice as a weed, its genetic similarity to rice, and its potential use as a model for cereal crops.

The objectives of this study were to use DNA microsatellite marker techniques to distinguish among red rice types, rice cultivars, and red rice–cultivated rice hybrid derivatives (RC hybrids) and to assess the genetic variation among pop-

ulations of these three *Oryza* groups in the southern United States.

Materials and Methods

From 1994 to 1999, seeds from diverse groups of mature red rice plants were collected from plants growing in rice fields in Arkansas or were acquired from independent sources. Generally, seeds were initially collected from individual panicles or plants and designated as a particular accession (detailed descriptions and small samples of these accessions are available upon request from the corresponding author). During each of these years, a red rice nursery containing up to 160 entries was established in hill or row plots at Stuttgart, AR. The soil type at this location was a Crowley silt loam (fine, montmorillonitic, thermic Typic Albaqualf). Standard field preparation and crop production practices were followed (Helms and Slaton 1994). Off-types were rogued to ensure phenotypic uniformity and minimize genetic diversity within each accession. Seeds were harvested for increase and planted the following year.

In 1999 a nursery consisting of 10 seeds per entry was planted in hills (spaced approximately 1 m apart) and grown to maturity as before. Seeds of 89 representative red rice accessions from this nursery, including 10 known or putative RC hybrids, were selected for DNA microsatellite marker analysis. The nomenclature system for these red rice types and RC hybrids consisted of a two-letter prefix indicating the state of acquisition (e.g., AR, LA, MS, or TX for Arkansas, Louisiana, Mississippi, or Texas, respectively) followed by a numeric code. Red rice types were classified phenotypically as awned or awnless, with glume colors of "strawhull" (SH) or "blackhull" (BH). All had red seed coats. Seven rice cultivars (awnless, SH types with light brown seed coats) were included as standards. These included three long-grain, tropical japonica cultivars from Arkansas: Kaybonnet (KBNT), Katy (KATY), and Starbonnet (STBN); two tropical japonica cultivars from Louisiana: Bengal (BNGL), a medium grain, and Cypress (CPRS), a long grain; and one tropical japonica cultivar from Texas: Gulfmont (GFMT). The only temperate japonica cultivar included was 'L201', a long grain from California (Mackill 1995). Four known F4 RC hybrids (obtained by hand-crossing followed by three generations of selfing) (Oard et al. 2000) and six putative RC hybrids were also included. The known RC hybrids were LA 1997-49 (BNGL × LA-Hope#8, a BH awned red rice), LA1997-48 (LA-Hope#8 × BNGL), LA1997-47 (SH awnless red rice × transgenic, glufosinate-resistant CPRS line, CPB6A), and LA 1997-50 (CPRS × LA-Hope#8). Both RC hybrids derived from BNGL were medium-grain types, and both RC hybrids derived from CPRS were long-grain types. All known and putative RC hybrids were classified as awnless, strawhull types.

DNA Isolation

Fifteen seeds from each of the 89 red rice entries and seven cultivated rice types were sown on moistened paper towels in disposable petri dishes and germinated at 30 C. Seedlings were harvested after 7 to 10 d, frozen with liquid nitrogen, and stored at −80 C. The number of germinated seeds ranged from 4 to 15. Seedlings were ground in liquid nitrogen and the total genomic DNA extracted using a procedure previously described, with minor modifications (Tai and Tanksley 1990). Five milliliters of preheated (65 C) extraction buffer (100 mM Tris–HCl pH 8, 50 mM ethylenediaminetetraacetic acid [EDTA] pH 8, 500 mM NaCl, 1.25% sodium dodecyl sulfate [wt/v], 0.0083 N NaOH, 0.38 g sodium bisulfite per 100 ml buffer) was added to each tissue sample with thorough mixing. After incubation at 65 C for about 30 min with periodic mixing by inversion, 1.54 ml of 5 M potassium acetate was added to each sample and mixed. Samples were then incubated on ice for about 1 h, followed by centrifugation at 2,300 × *g* for 15 min at 4 C. Supernatants were filtered using miracloth[1] and DNA precipitated using 0.67 volumes of 2-propanol. After an overnight incubation at −20 C, the samples were centrifuged at 2,300 × *g* for 20 min at 4 C. Pellets were suspended in 400 μl of TE (10 mM Tris–HCl, 1 mM EDTA, pH 8) and subjected to a second precipitation using 0.1 volumes of 3 M sodium acetate and 0.67 volumes of 2-propanol. After centrifugation in a microfuge at 28,400 × *g* for 5 min at room temperature, supernatants were decanted and rinsed with 70% ethanol and air-dried. DNA pellets were suspended in 200 μl of TE and quantified by agarose gel electrophoresis and staining with ethidium bromide. Undigested λ DNA was used as a concentration standard. DNA samples were adjusted to a concentration of 10 to 20 ng μl^{-1}.

TABLE 1. Microsatellite markers used for DNA fingerprinting of red rice and cultivated rice.[a]

Microsatellite rice marker	Chromosome number	Chromosome arm of DNA marker	Number of alleles observed
RM 220	1	Short	6
RM 212	1	Long	4
RM 53	2	Short	5
RM 251	3	Short	6
RM 261	4	Short	5
RM 241	4	Long	7
RM 13	5	Short	5
RM 26	5	Long	3
RM 253	6	Short	7
RM 234	7	Long	8
RM 230	8	Long	2
RM 215	9	Long	5
RM 219	9	Long	12
RM 258	10	Long	3
RM 167	11	Short	5
RM 206	11	Long	6
RM 19	12	Short	9
RM 235	12	Long	6

[a] Markers that were not scorable included RM 30 (6 long), RM 38 (8 short), RM 49 (3 long), RM 82 (7 short), RM 216 (10 short), RM 221 (2 long), and RM 231 (3 short).

Microsatellite Marker Analysis

Twenty-five microsatellite markers (also called "simple sequence repeat" markers or "SSRs") were selected for their dispersion across the rice genome (Table 1). Primers for these markers were obtained commercially.[2] Polymerase chain reaction (PCR) amplification reactions were performed as described by Chen et al. (1997), with minor modifications. Briefly, 15 μl reactions containing 0.2 μM prim-

ers, 200 μM deoxyribonucleotides, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 20 ng of template DNA, and 0.25 units of *Taq* polymerase were subjected to the following PCR profile: 94 C for 5 min; 35 cycles of 94 C for 30 s, 55 C for 30 s, and 72 C for 30 s; 72 C for 5 min; hold at 4 C. The resulting products were fractionated by size on 4% polyacrylamide gels and stained with silver (Panaud et al. 1996).

Analysis of Genotypic Data

Genotypes were determined for each accession at each of the 25 microsatellite loci. Genetic distance (GD) between all accession pairs was calculated as $GD = 1 - A/N$, where A is the total number of microsatellite alleles shared by two accessions and N the total number of microsatellite loci scored between the two accessions. GD values can range from zero (all alleles in common) to unity (no alleles in common). The matrix of GD values was subjected to hierarchical cluster analysis, using the average linking method to determine natural grouping among the accessions. A cluster was defined as a group of accessions with an average GD among its members that was lower than the overall average GD and if the average GD between the new cluster and its most related cluster was greater than the overall average GD. GDs were calculated with an SAS IML program, whereas clustering was performed with PROC CLUSTER of SAS (SAS 1999).

The GD matrix was also subjected to a multidimensional scaling (MDS) analysis. MDS is similar to principal coordinate analysis, as both start with a dissimilarity matrix and use eigen analysis to summarize and condense variance within the GD matrix into few dimensions. MDS then uses an iterative process to find a set of coordinates in Euclidian space that best represent the original distances in the GD matrix (Gizlice et al. 1996). Thus, the linear distance between two points on an MDS plot estimates the actual GD between those two points. MDS analysis was performed using SAS software (SAS 1999). Allele frequencies were calculated for all accessions as well as for each of the four groups: awnless red rice, awned red rice, accessions that clustered with the cultivars (which included two RC hybrids), and known and putative RC hybrids (except the two that clustered with the cultivars). Allele frequencies for the red rice (both awned and awnless) entries were also calculated.

Results and Discussion

Genetic variation among accessions in this study was assessed using microsatellite markers. These markers are robust, codominant (i.e., they can detect heterozygous loci), exhibit high allelic variation, and are widely distributed throughout the *Oryza* genome (McCouch et al. 1997). More than 500 microsatellite markers have been characterized in rice to date (S. McCouch 2001). Given their codominant nature, microsatellites were also useful in identifying RC hybrids.

Typically, individual red rice entries were homozygous for any given marker (loci), yielding only one allele; however, in rare cases an individual accession was heterozygous at a particular locus, producing two alleles (Figure 1). The total number of alleles per microsatellite locus among all entries ranged from 2 to 12 (Table 1). Of the 25 loci examined, only 18 yielded amplification products that could be reliably scored (Table 1). Only data from these 18 loci were used in subsequent analyses.

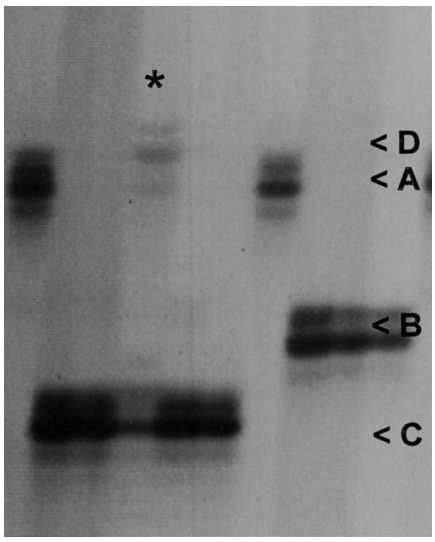

FIGURE 1. Panel of silver-stained polyacrylamide gel showing four *Oryza* alleles (A, B, C, and D) detected by the microsatellite marker RM 220. Entries shown in this example are (left to right) KatyRR putative RC hybrid, KATY cultivar, LA 1997-48 (BH awned red rice × BNGL RC hybrid), LA 1997-47 (SH awnless red rice × CPRS RC hybrid), AR 1997-46 (SH awned red rice), L201 cultivar, AR 1997-32 (SH awnless red rice), and AR 1997-31, AR 1997-16, and AR 1997-15 (all BH awned red rice). The SH awnless red rice × CPRS RC hybrid in lane four (*) had two alleles (C and D), indicating that it is heterogeneous at this locus. All other entries shown on this panel produced a single allele, indicating that they are homozygous at this locus. Generally for RM 220, awnless red rice accessions produced allele A, awned accessions produced allele B, rice cultivars produced allele C, and hybrids produced allele A or C. RC hybrid, red rice–cultivated rice hybrid derivative.

GD averaged 0.63 among all accessions (Table 2) and ranged from 0 to 1 between pairs of accessions. Cluster analysis suggested that there were three distinct genotypic groups. Group 1 consisted of awnless, SH red rice types, with the exception of one accession with very short awns (MS1996-6; the same as SHA+ from a Mississippi red rice collection; DoLago 1982) (Figure 2A). Group 2 consisted primarily of awned, BH red rice (Figure 2A). The RC hybrid BNGL × LA Hope#8 (LA1997-49) was also in Group 2. The third group was composed of RC hybrids and rice cultivars. By our definition of a cluster ("Materials and Methods" section), these accessions formed one cluster, but there appeared to be two distinct subgroups. One subgroup consisted entirely of known or putative RC hybrids (Group 3A), and the other consisted of rice cultivars and two RC hybrids (Group 3B) (Figure 2A). The average GD between these two subgroups was 0.76. Most of the putative RC hybrids clustered closely with the known RC hybrids (Figure 2A), supporting the hypothesis that these were RC hybrids; however, the DNA markers used were not sufficient to definitively establish whether a particular red rice or cultivated rice accession was the progenitor of these putative RC hybrids. In addition, we need to genotype more rice cultivars to assist in determining the parentage of putative hybrids.

The pattern of genetic diversity observed in this study is consistent with the traditional classification of cultivated rice and red rice as the same species (sexually compatible) with differences in morphological characteristics such as hull col-

TABLE 2. Average genetic distance between and within groups of red rice, cultivated rice, and RC hybrids[a] as determined by cluster analysis.

General description of Group[b]	Group name	Average genetic distance
RC hybrids vs. cultivated rice	Group 3A vs. Group 3B	0.76
RC hybrids vs. awned red rice	Group 3A vs. Group 2	0.81
RC hybrids vs. awnless red rice	Group 3A vs. Group 1	0.62
Cultivated rice vs. awned red rice	Group 3B vs. Group 2	0.81
Cultivated rice vs. awnless red rice	Group 3B vs. Group 1	0.94
Awnless red rice vs. awned red rice	Group 1 vs. Group 2	0.84
Awnless red rice	Group 1	0.20
Awned red rice	Group 2	0.33
Cultivated rice	Group 3B	0.35
RC hybrids	Group 3A	0.63
All entries	All	0.63

[a] RC hybrids = hybrids of red rice and cultivated rice, including putative hybrids.
[b] In this table phenotypic descriptions of groups are generalized: one member of Group 1 has short awns, one member of Group 2 is an awnless, RC hybrid, and two members of Group 3B are RC hybrids or putative hybrids.

or and the presence or absence of awns. Random amplified polymorphic DNA (RAPD) analysis on a small subset of entries used in this study revealed similar cluster separation among awned and awnless red rice types as well as between a putative hybrid and cultivated rice (Estorninos et al. 2000). Genetic relationships among several "wild" and "weedy" *Oryza* species obtained from Malaysia and Suriname (not necessarily with red seed coats) (Vaughan et al. 1999) and Korea (Suh et al. 1997) have been evaluated using RAPD techniques. Other red rice accessions and related *Oryza* species were recently classified using marker techniques similar to those used in the present study (Vaughan et al. 2001). In that study, most SH awnless, SH awned, and brown or gold awned red rice accessions from the southern United States (including LA3, MS4, and AR1 [~ AR-StgS] from the present study) were genetically more similar to *O. sativa* ssp. *indica* ("tropical") rice than to *O. sativa* ssp. *japonica* ("temperate") rice, *O. nivara*, or the noxious weed, *O. rufipogon*. BH awned red rice accessions (including TX4 from the present study) were genetically similar to an accession of *O. rufipogon* (IRGC 10549) and somewhat less similar to *O. nivara*. The close relationship between BH red rice, *O. rufipogon*, and *O. nivara* based on microsatellite markers is not necessarily inconsistent with BH red rice belonging to *O. sativa* given the shared genetic background of these species and the fact that only 18 DNA markers were used in the Vaughan et al. (2001) study. Evolutionarily, *O. rufipogon*, a wild perennial species, is considered to be the progenitor of the wild annual species *O. nivara*, which itself is considered the progenitor of *O. sativa* (Singh and Khush 2000). *Oryza rufipogon* and *O. nivara* are among the several *Oryza* species in the AA genome group with *O. sativa* that can readily cross with *O. sativa* (Sitch 1990). Regardless of their species designation, red rice populations in the southern United States appear to be genetically diverse.

Allele Frequencies

The number of alleles per locus ranged from two to 12. In total, 102 alleles were scored (average of 5.7 alleles per locus). The accessions in Group 1 (primarily awnless SH red rice types) had 45 alleles, including 10 that were not present in the other three groups. Accessions in Group 2 (primarily awned BH red rice types) had 56 alleles, including 10 that were not present in the other three groups. Based on this sample, the awned BH red rice accessions appeared more diverse than the awnless red rice accessions. This is supported by the higher average GD among the awned (0.33) than among the awnless (0.20) red rice types (Table 2).

Accessions in Group 3B (primarily cultivars) had 44 alleles, including 10 that were not present in the other three groups, whereas the RC hybrids of Group 3A had 58 alleles, 11 of which were unique. Although Groups 3A and 3B consisted of few lines, their diversity was quite high. This is reflected in the high average GD observed within Group 3B (0.35) and Group 3A (0.63). Higher diversity would be expected in RC hybrids because RC hybrids would sample alleles from rice cultivars and red rice types.

Using Microsatellite Markers to Distinguish Between Red Rice and Rice

These results suggest that microsatellite markers developed for *O. sativa* could be used to distinguish between red rice and cultivated rice. Of the 18 markers used, four (RM 215, RM 234, RM 251, and RM 253) produced one or more alleles that were found only in red rice or cultivated rice. In addition, awnless red rice types were genetically unique from the seven rice cultivars at the RM 230 locus.

Markers used in this study provided genotypic profiles for CPRS and GFMT that were indistinguishable (except at RM 219 where CPRS produced amplification products, but GFMT did not). A small subset of these markers provided unique identification among CPRS or GFMT and the remaining five cultivars. For example, RM 219 by itself provided unique profiles for BNGL, CPRS, KATY, and STBN. RM 219 in combination with RM 215 (both located on chromosome #9) provided unique identification among the six cultivars.

Microsatellite markers used in this study were capable of distinguishing between temperate japonica and tropical japonica cultivars as well as between medium-grain and long-grain temperate japonica cultivars. Previous work with RAPD markers provided similar results (Mackill 1995). The

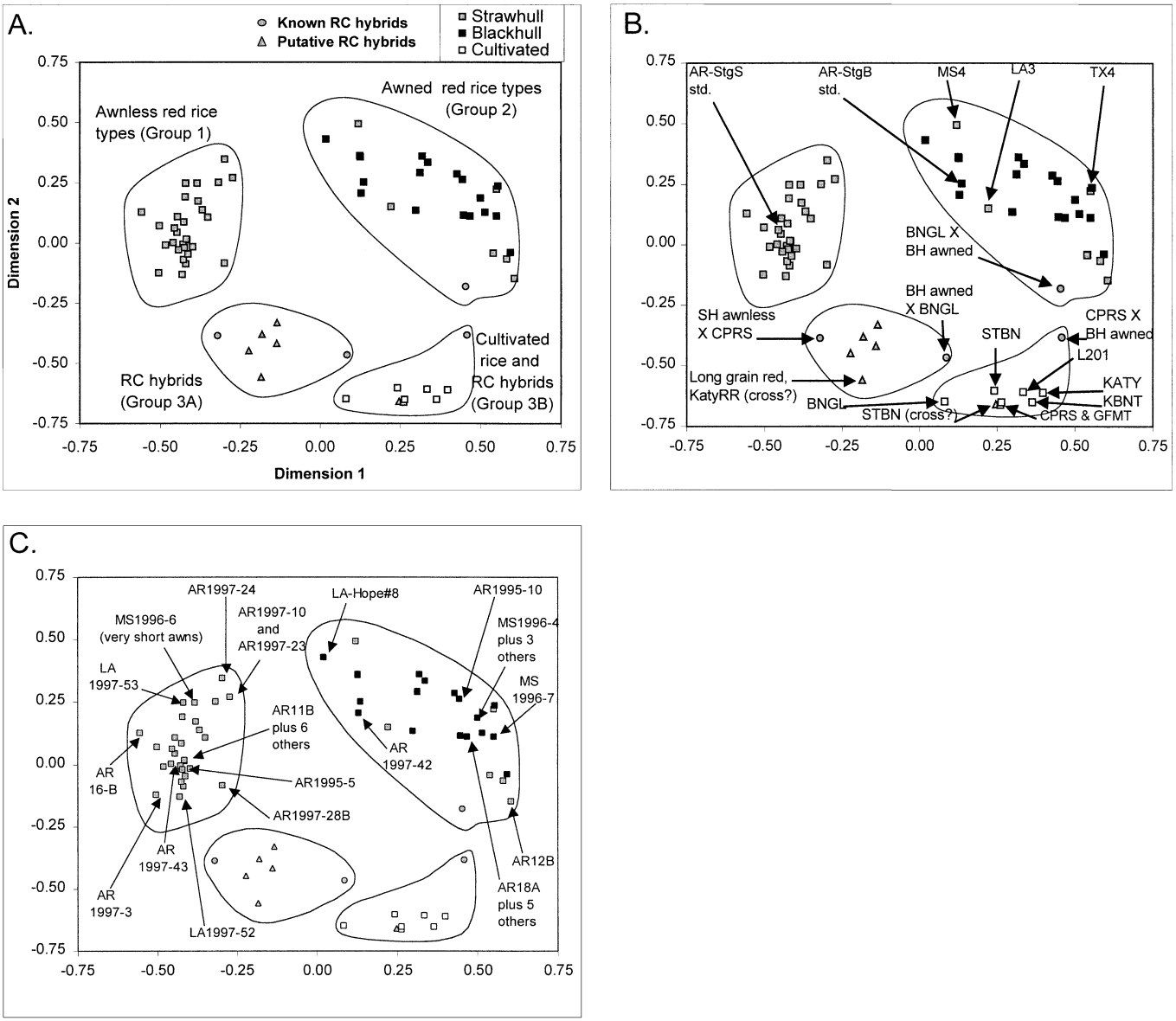

FIGURE 2. (A) Multidimensional scaling (MDS) analysis of the genetic distance (GD) among red rice, rice cultivars, and red rice–cultivated rice hybrids (RC hybrids). The linear distance between two points estimates the actual GD between the two accessions (i.e., accessions that are close have low GD between them, whereas accessions that are far apart have a large GD). Accessions that were grouped together in the cluster analysis are circled. (B) MDS data identical to those in (A) but with labels for selected rice cultivars, red rice standards, and RC hybrids. (C) MDS data identical to those in (A) but with labels for selected red rice entries with either rare genetic profiles or shared (identical) genetic profiles. All entries in Groups 3A and 3B are awnless.

only temperate japonica cultivar in our study (L 201) was distinguishable from six tropical japonica cultivars with marker RM 53. The only medium-grain cultivar in the study (BNGL) was distinguishable from six long-grain cultivars with marker RM 234 or RM 251.

Several red rice accessions had extremely rare alleles (not shown). These accessions included AR1997-43, LA-Hope#8, AR16B, AR1997-3, AR1997-42, and AR-StgB at the RM 234 locus; LA-Hope#8 and LA1997-53 at the RM 253 locus; AR1997-5 and LA1997-52 at the RM 167 locus; AR16B and LA1997-52 at the RM 219 locus; MS1996-4 and MS4 at the RM 235 locus; and AR17D and AR17C at the RM 261 locus (accessions labeled in Figure 2B or 2C). These results demonstrated the potential of microsatellite markers for identifying individual red rice types within diverse populations.

There were 48 distinct genetic profiles identified among red rice accessions in this study; however, several accessions had microsatellite profiles identical (based on the markers used in this study) to at least one other accession. The number of red rice accessions with identical microsatellite profiles ranged from two to seven. Most accessions with identical profiles were sampled from the same rice field or from the same farm (Figure 2C). Identical accessions AR18A, AR18E, AR1997-15, AR1997-16, AR1997-21, and AR1997-31 were sampled from two farms in Arkansas County, AR. In other cases, identical accessions were obtained from locations that were many kilometers apart. Identical accessions AR11B and AR11H were obtained from one field, whereas AR13C, AR15A, AR16E, and AR17A were each collected from a different field in Arkansas County, AR. Another identical type, AR1995-5, was obtained

approximately 200 km to the north in Greene County, AR. The identical accessions AR10A and AR11A were collected in Arkansas County, AR, whereas accessions with identical microsatellite profiles, MS1996-3 and MS1996-4, were obtained from Mississippi. Although identical microsatellite profiles were discovered in accessions obtained at great distances from one another, this does not mean that the accessions themselves were genetically identical but rather that the accessions were indistinguishable from one another based on markers representing a very small fraction of their total DNA.

Using Microsatellite Markers to Evaluate RC Hybrids

The markers used in this study separated all the 10 known or putative RC hybrids from red rice and rice cultivars in this study, and each RC hybrid was distinguishable from all other RC hybrids. GD data revealed interesting insights into the parentage of several putative RC hybrids. Putative RC hybrid MS1996-8 was most closely related to GFMT (0.06), CPRS (0.12), and STBN (0.13) and less closely related to BNGL (0.56), KATY (0.29), KBNT (0.32), and L201 (0.41) (data not shown). This is consistent with historical accounts that this long-grain red rice resulted from a cross between red rice and STBN. It was discovered originally in a field of STBN rice in Mississippi and was phenotypically similar to STBN except for its red grains (B. Keith, personal communication). The GD between MS1996-8 and STBN is very low, suggesting that the initial RC hybrid may have then crossed to STBN, resulting in a red rice accession that is 75% STBN. The putative RC hybrid KatyRR was discovered originally in a field of KATY in Stuttgart, AR, in 1994 (K. Moldenhauer, personal communication) and was more closely related to GFMT (0.53), STBN (0.59), and CPRS (0.56) than to KATY (0.61) (detailed data not shown), suggesting that KATY is no more likely than these other cultivars to be a parent of KatyRR.

Several microsatellite markers produced alleles that were rare among red rice accessions but common in cultivated rice (allele data not shown): RM 53 produced these rare alleles in red rice accessions AR1995-5, AR1997-28B, and AR12B; RM 167 produced such alleles in AR1997-10, AR1997-23, and AR1997-24; RM 235 produced a rare allele in AR1995-10; RM 13, RM 216, and RM 219 produced rare alleles in MS1996-7; and RM 19 produced a rare allele in TX4 (red rice accessions labeled in Figures 2B and 2C). This suggests that the red rice accessions sharing the same rare allele may have a recent common ancestor and may possibly be derived from matings between red rice and RC hybrids.

Microsatellite marker analysis used in this study separated rice cultivars and red rice populations. Several microsatellite alleles were unique to red rice accessions or to rice cultivars. These alleles could be useful in studying red rice biology, dispersion of red rice genotypes, and degree of hybridization between red rice and cultivated rice. Accurately determining the cultivated rice parent of a putative RC hybrid will require genotyping more rice cultivars and perhaps using more microsatellite markers.

Finally, cultivated rice lines with red bran color belonging to the AA species *O. sativa*, *O. glaberrima*, *O. nivara*, *O. rufipogon*, and four others account for about one-sixth of the ~18,000 *Oryza* entries in the U.S. rice collection (GRIN 2000). Microsatellite marker analyses comparing the genetic relationships between these lines and weedy U.S. red rice types may provide additional insights into the origins of red rice and cultivated rice populations in the southern United States.

Sources of Materials

[1] Miracloth, Calbiochem, P.O. Box 12087, La Jolla, CA 92039-2087.

[2] Primers, Research Genetics, 2130 Memorial Parkway, Huntsville, AL 35801.

Acknowledgments

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